

Carbon-Water-Nitrogen relationships between lichens and the atmosphere: Tools to understand metabolism and ecosystem change

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Abstract

Due to the close linking between the biosphere and atmosphere, there are clear impacts of changes in climate, atmospheric deposition of nutrients/pollutants and land use (Global Changes) on the terrestrial biosphere. Lichens, with a direct dependence on atmospheric conditions, are much more affected by their immediate microclimate than by the ecosystem's prevailing macroclimate. In contrast to higher plants, poikilohydric organisms have different mechanisms of water and CO₂ exchange. The application of stable isotopes to the understanding of the mechanisms that are fundamental to lichen gas exchange and water uptake is a promising tool for the evaluation of lichen response to environmental changes. Indeed, lichens have been shown to be influenced by a large number of natural and anthropogenic environmental factors, serving as ecological indicators. Thus, we may use these organisms to model the impact of key global change drivers, such as nitrogen deposition and biodiversity changes, at local scale. Particularly useful is the application of the Lichen Diversity Value (LDV) in order to evaluate the impact of global drivers. Moreover, it has been shown that these indices, associated with main photobiont types, green-algae (LDV_{ch}) or cyanobacteria (LDV_{cyh}), and/or nitrophilous versus oligotrophic species, were good candidates as ecological indicators. Besides mapping with high spatial resolution the effects of climate alterations, lichen functional groups could also be used as an early-warning system in order to detect the first effects of climate change in ecosystems before sudden shifts occur on other components that may be less sensitive. Clearly, lichens possess the adequate traits to be used as powerful indicators of complex interactions between atmosphere and biosphere, and thus can generate potentially interesting models for global change drivers.

Key words

Climate change, Ecology, Photobionts, Physiology

Why do we need to understand biosphere-atmosphere interactions?

The biosphere has a significant impact on the composition of the atmosphere, *and the interaction between biosphere and atmosphere affects all living organisms, including humans*. Changes in climate, atmospheric deposition of nutrients/pollutants and land use (Global Changes) have observable impacts on the terrestrial biosphere (i.e. IPCC 2007; Rockstrom et al. 2009; Scheffer et al. 2009). It is relevant to quantify global change effects on ecosystem C/N stocks and exchange processes between the terrestrial biosphere (Canadell et al. 2000), the atmosphere and hydrosphere with a specific focus on C and N trace gases. In the near future it is crucial to understand the complex feedback mechanisms between the biosphere (e.g. biodiversity patterns) and atmosphere, under changing environmental conditions (IGBP 2007; Steffen et al. 2004).

Given the close linking between the biosphere and atmosphere, the exchange of carbon (CO₂) and water vapor between biosphere and atmosphere, and the deposition of nutrients and heavy metals to the plant or ground surface are very important areas of research. Research in this area involves measurements of the exchange of gases (i.e. CO₂ and water vapor) using eddy covariance (and other) flux techniques, spectroscopic and tracer release methods (for trace gases such as N₂O), as well as satellite data and modeling approaches (i.e. Canadell et al. 2000). Thus, studying water and carbon fluxes can generally contribute to a better understanding of ecosystem functioning and biodiversity response to global change. Stable isotopes are currently used to investigate biosphere/atmosphere exchange processes and mechanisms at different spatial (from plant to ecosystem) and temporal (from short- to long-term responses) scales (Bowling et al. 2008; Bruggemann et al. 2011; Dawson et al. 2002; Unger et al. 2010, 2012).

Approximately 8% of the earth's land surface is covered by vegetation types dominated by lichens (Büdel et al. 2000), especially in environments with limited nutrition or water supply (Lange et al. 1992; Belnap et al. 2001). Any poikilohydric ground cover mediates the exchange of evaporative water between the pedosphere and atmosphere. Lichens potentially use a wide range of water sources such as soil water, precipitation, dew, fog or, in the case of some mosses and green algal lichens, even water vapor (e.g. Lange et al. 1988). Hence, these organisms are much more affected by their immediate microclimate than by the ecosystem's prevailing macroclimate. In contrast to higher plants, poikilohydric organisms have different mechanisms of water and CO₂ exchange. Thus, studying lichens as a globally important ground cover component and a physiological model of poikilohydric organisms may provide the scientific community with new insights of pedosphere-atmosphere exchange processes.

The importance of “fingerprinting” to study biogenic fluxes between lichens and the atmosphere

All environments with a scarcity of nutrients or water supply are dominated by poikilohydric cryptogams such as bryophytes (mosses and liverworts) and lichens. Over 33% of the Earth's surface is covered by semiarid and arid lands dominated by biological soil crusts composed mainly of cyanobacteria and lichens (Belnap et al. 2001). Even in forest ecosystems, lichens can sometimes constitute up to half of the above ground biomass. Accordingly, in these ecosystems poikilohydric organisms have a large effect on water and CO₂ fluxes. Poikilohydry determines the physiology and ecology of cryptogams, the water status of which is completely dependent on the environment, reaching equilibrium with the atmosphere (e.g. Green and Lange 1994). Moreover, there is increasing evidence that morphology and structure of the thallus of these organisms may be very important in determining CO₂ uptake rates and gas exchange in general (Máguas and Brugnoli 1996; Máguas et al. 1997; Lakatos et al. 2007; Hartard et al. 2008; Larsson et al. 2012).

The use of stable isotopes is a powerful research tool in environmental sciences. Its combination with: i) concentration measurements (providing “Keeling-type” plots; Keeling 1960) and ii) flux measurements, allow separation of net CO₂ exchange into photosynthetic and soil respiration components, and the evapotranspiration flux into soil evaporation and leaf transpiration (Yakir and Wang 1996). These and similar approaches help to define various fluxes in phanerogam dominated-ecosystems (Yakir and Sternberg 2000), and we may also use these techniques to investigate the origin of biogenic fluxes through a “molecular fingerprinting” approach. Carbon and oxygen isotopic composition are of major importance in evaluating physiological processes driving CO₂ and water exchange because they provide information on fractionation processes as well as characterize plant metabolism and interactions with the ecosystem (i.e. Dawson et al. 2002).

Due to their poikilohydric nature and the lack of stomatal control, lichens do not show the typical stable isotope compositions (d¹³C) of higher plants. The d¹³C value of their organic matter (OM) vary between -12 and -23 ‰, and such a high d¹³C heterogeneity is thought to be due to discriminating factors such as the CO₂ diffusion resistance and CO₂ source, further than photosynthetic RuBisCO (Ribulose-1,5-bisphosphate carboxylase oxygenase) fractionation (Máguas and Brugnoli 1996; Máguas et al. 1997; Lakatos et al. 2007). Indeed, d¹³C values of lichens result from a combination of species-specific differences in resistances to inward CO₂ fluxes (Máguas and Brugnoli 1996; Máguas et al. 1997), CO₂ source signature from the substratum (e.g. soil and bark) (Lakatos et al. 2007) and the CO₂-fixation mechanism of their photobiont (Máguas et al. 1993, 1995; Smith and Griffiths 1996, 1998; Smith et al. 1998) (Fig. 1). Recent studies confirm that two main categories of lichen are identifiable (Lakatos et al. 2007), depending on the existence or absence of a CCM (CO₂-concentrating mechanism) in the primary photobiont, producing a significant

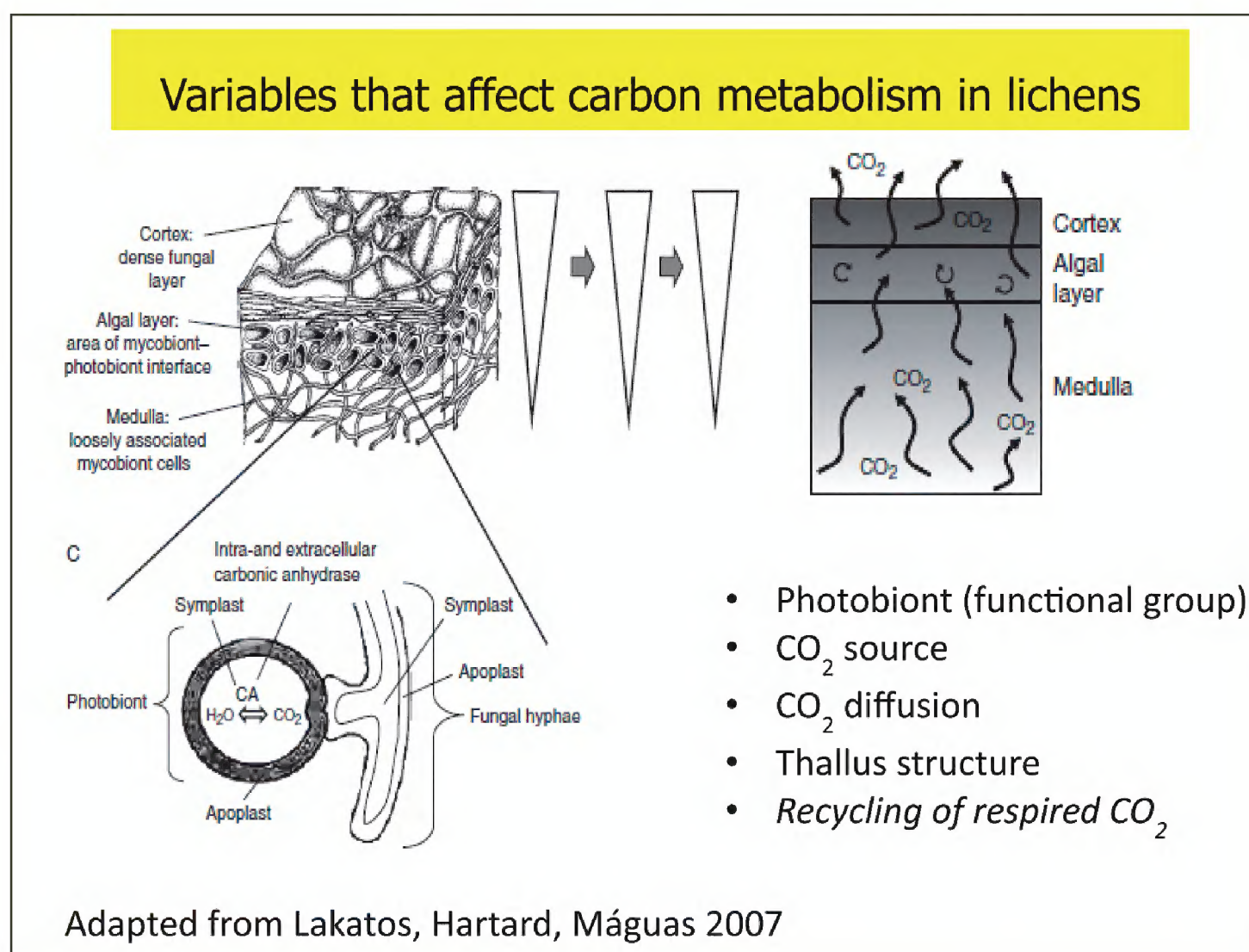


Figure 1. Schematic illustration of a cross-section through a lichen thallus with distinctive cortex, algal layer and medulla and the main factors contributing to thallus CO₂ exchange and the consequence for carbon d¹³C fractionation that affects organic matter in lichens.

difference of on average 10 ‰ on d¹³C values between the two groups (Fig.1). Cyanobacterial or green algal photobionts with CO₂-concentrating mechanisms increase the internal carbon pool near the carboxylation site of RuBisCO (Palmqvist et al. 1994a, b). As a consequence of the increased substrate availability, the rate of carboxylation is increased while that of photorespiration is decreased. Moreover, the CCMs tempers the effect of CO₂ diffusion within the thallus, e.g. at high water contents or super saturation (Máguas et al. 1997). Thus, d¹³C values of CCMs-containing lichens vary between -16 ‰ and -26 ‰ with a mean around -22‰. In tripartite lichens with two photobionts, (a primary green algal photobiont without a CCM and a secondary cyanobacterial photobiont in specialized thallus structures called cephalodia) the organic δ¹³C values are determined by the green algal partner (Máguas et al. 1995; Green et al. 2002), since the main function of the cyanobionts is N fixation. Thus, OM of these tripartite lichens is characterized by d¹³C similar to C3-plants (mean of 18 species -32 ± 1.6 ‰; n = 43) since their green algae, such as *Myrmecia*, *Dictyochloropsis* (Smith and Griffiths 1996) and *Coccomyxa* (Palmqvist et al. 1994b), lack both pyrenoids and CCMs. Similar values can be found in lichens with only one photobiont: e.g. the CCM-lacking green algal genus *Trentepohlia* (Lakatos et al. 2006), which is the pre-

dominant photobiont in the tropics and subtropics (Sipman and Harris 1986). Thus, the presence or absence of a CCM is the principal explanation for the difference in $\delta^{13}\text{C}$ values between two different functional groups.

Over the surface of a single thallus CO_2 diffusion resistances can be modified by morphological structures such as the conglutinated cortical layers, thallus thickness and density and concomitant structural changes during water absorption. Hence, thinner thallus regions such as margins or tips, which are often loosely constructed and displaying lower CO_2 diffusion resistances, should also lead to lower $\delta^{13}\text{C}$ discrimination (depleted $\delta^{13}\text{C}$). However, the observed high heterogeneity of $\delta^{13}\text{C}$ of margins *versus* center parts within different growth forms and photobiont groups (from 0.25 to 2.5 ‰) does not give a clear indication of the expected discrimination factor associated with morphology and thallus structure, and no correlation with respect to growth form has been assessed (Lakatos et al. 2007).

Besides the major photosynthetic fractionations due to the transport and fixation of CO_2 , one crucial factor influencing $\delta^{13}\text{C}$ is the origin of the carbon source used by lichens in a specific microenvironment. Several factors may contribute to this: i) depending on where they are located lichens can be attached to a substratum where the most direct CO_2 source is not atmospheric CO_2 ; and ii) different lichen species or different individual thalli might fix respired CO_2 from different ecosystem components. For example, it is well established that in macrohabitats such as closed forests, the source of ambient CO_2 gradually changes with height from the forest floor to the canopy (the vertical CO_2 profile) (e.g. Sternberg 1989; Buchmann et al. 1997). Moreover, CO_2 derived from different respiring substrata (e.g. soil, tree bark, leaf) has a $\delta^{13}\text{C}$ signal that is more depleted than that of ambient air.

As mentioned earlier, poikilohydric cryptogams can have a major influence on water and CO_2 fluxes in an ecosystem but their mechanisms of water exchange differ to those of higher plants. Their water status, for example, varies passively with surrounding environmental conditions, and they have neither a continuous influx of water nor stomata to control water deficit. Hence, during evaporation no isotopic steady state can be achieved and the $\delta^{18}\text{O}$ composition of both the thallus water and the evaporated water is expected to show progressive enrichment similar to the Rayleigh distillation process. As the water also transduces its oxygen isotopic signal to CO_2 *via* hydration of dissolved CO_2 (e.g. Amundson et al. 1998; Tans 1998; Stern, et al. 1999), respired CO_2 should also reflect the oxygen isotopic composition of the thallus water. Thus, the isotopic composition of the thallus water of lichens growing in their natural habitat appears to depend mainly on two separate factors: the isotopic signal of the predominantly available water source and the water potential difference between the thallus and the surrounding air (Hartard et al. 2008, 2009). From this, it follows that $\delta^{18}\text{O}$ values of lichens which are already in 'steady state', i.e. in continuous equilibrium with its surroundings, reflect the isotopic signal of the atmospheric vapor. In contrast lichens close to physical equilibration with their surroundings will approach isotopic equilibration with the surrounding isotopically lighter water vapor.

As a model organism, the globally distributed lichen *Cladina arbuscula* was studied under laboratory conditions as well as in the field. During a desiccation experiment, $\delta^{18}\text{O}$ values of thallus water and respired CO_2 became enriched by $\sim 7\text{‰}$ and followed an enrichment pattern similar to that of higher plants. However, the observed degree of enrichment was lower in comparison to higher plants due to (i) the lichen's inherent lower evaporative resistances and (ii) a stronger effect of the more depleted surrounding water vapor (Hartard et al. 2008, 2009). In the same species, when growing in its natural habitat, $\delta^{18}\text{O}$ values of thallus water principally proved to be highly depleted and strongly depended on the absorption of water vapor. Moreover, the results indicated that lichen cushions substantially reduce soil evaporation rates which may enhance their distinctive isotopic contribution to ecosystem water fluxes (Hartard et al. 2008, 2009). Therefore, the data indicate a strong influence of poikilohydric ground cover on soil evaporative fluxes, especially during drier periods without rain. During these periods, lichens predominantly utilize the more depleted air moisture and, hence, also evaporate more depleted water vapor into the atmosphere.

The use of lichens as ecological-indicators of biogenic fluxes between biosphere and the atmosphere

Lichens, which rely largely on the atmosphere for water and nutrient supply, can be used as suitable indicators of environmental changes in terrestrial ecosystems (Will-Wolf et al. 2006, Bergamini et al. 2005). However there are several concerns, such as to the type of measures that can be made with lichen communities and the spatial-temporal scales that may be studied. Recently, a standardized methodology has been established for collecting lichen diversity and abundance data in the field; this is the European Method (Asta et al. 2002). This methodology is based on a 50x10cm sampling grid, divided into 5 squares, that is placed on the four main aspects of tree trunks (Fig. 2). All lichen species occurring in the grid are identified and the number of squares occupied by each species that occurs on each tree is recorded as its frequency. From these data one can calculate two main variables: total species richness and total Lichen Diversity Value (LDV) (Fig. 2). The LDV is the sum of the frequency of all species on each tree, divided by the number of trees sampled. Geostatistical models are used to predict the spatial structure of the data, and ultimately interpolate the variable values in ensample locations using kriging (Fortin and Dale 2005). This process is based on variography analysis (Mitchell et al. 2000).

An alternative to total diversity is to use of functional traits, such as lichen photobiont type, eutrophication and water stress tolerance, which is strongly involved in species responses to several environmental factors. Indeed, in a study conducted in a Mediterranean *Quercus faginea* subsp. *broteroi* forest, the lichen functional diversity was highly significantly related to potential solar radiation, an integrated measure of long-term microclimate. The green-algal lichens were positively related to potential solar radiation whereas cyanolichens were negatively related (Pinho et al. 2010). In this study it has been proved that lichen communities can be used to: i) model long-term microclimatic conditions with high spatial resolution and ii) model the disturbance caused

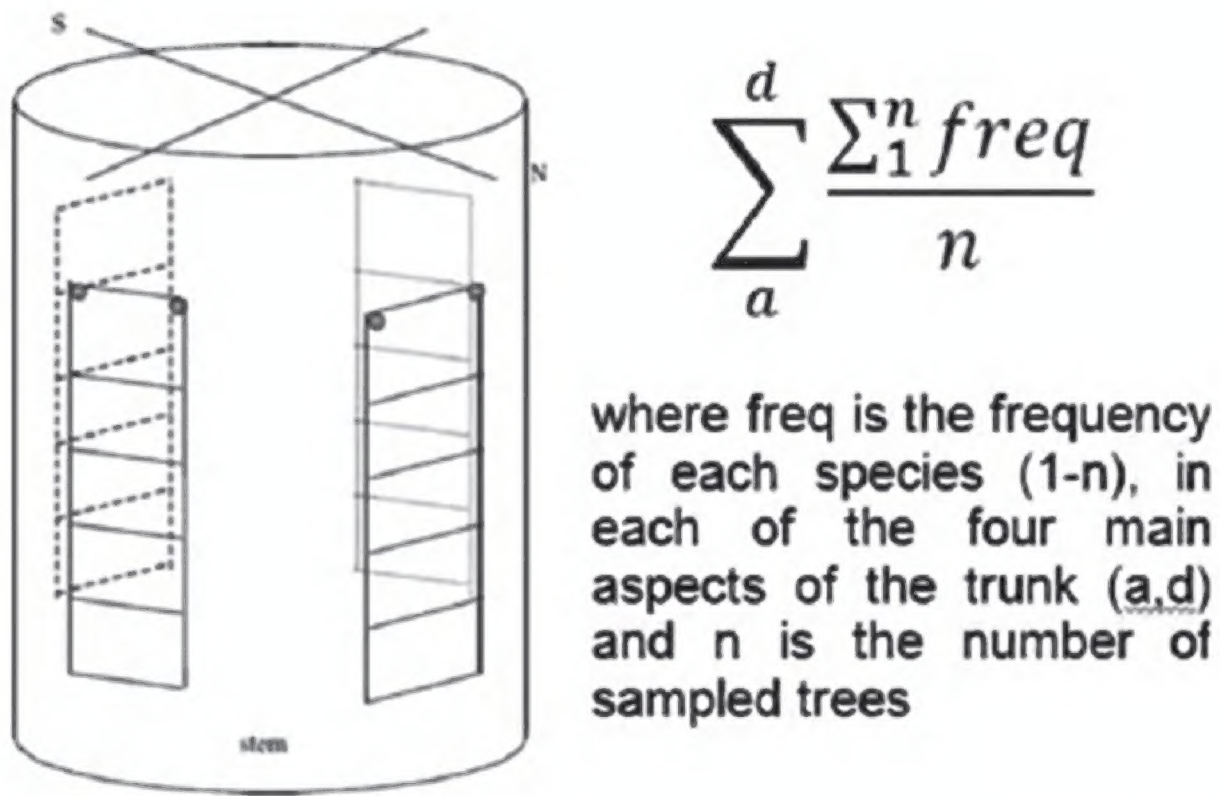


Figure 2. Distribution of the sampling grid in a tree trunk, original picture (Asta et al. 2002)

by neighborhood areas, even in natural parks with low-intensity human activities, for conservation purposes (Pinho et al. 2011, 2012a, b).

Lichens can also be classified according to their preferences or tolerance to eutrophication (Nimis and Martellos 2008). Several authors have pointed out that nitrogen from agricultural origin is playing an increasingly important role in changing lichen communities (Hultengren et al. 2004; Purvis et al. 2003). The results observed in an ecosystem oak woodland in southern Portugal were in agreement with the suggestion that the alteration induced by humans in the N cycle is among the most important drivers associated with global changes (Rockstrom et al. 2009). In this work, the dispersion and the distance of influence of eutrophication/ NH_3 in lichens was found to be of short-range. However, the areas affected by it were found to be widespread in the territory, and always associated to agricultural sources (Pinho et al. 2011, 2012b).

Conclusions

Since lichens are directly influenced by microclimatic conditions, such as light, water, temperature and CO_2 concentration, the isotopic composition of their OM integrates environmental factors acting on their specific microhabitat over a range of weather conditions, as well as a variety of land-uses over long periods of time. The isotopic composition of OM is determined by an economic equilibration between carbon source and sink, which are mainly photosynthesis and respiration. Although the mycobiont dominates the OM pool, the carbon acquisition of the lichen depends

on the water content, light intensity and CO₂ fixation of the photobiont. Carbon isotope discrimination processes of ¹³C can thus be related to CO₂ acquisition modes, CO₂ diffusion and CO₂ sources. To summarize, in several microhabitats, respired d¹³C-depleted CO₂ serves as the carbon source for photosynthesizing lichens, thus biasing their characteristic isotopic signatures, which otherwise are determined by physiological processes. Thus, lichens can be used as tracers to point out the prevailing CO₂-sources in microhabitats. Therefore lichen OM will also indicate, especially in the younger thallus parts, an alteration in ambient ¹³C-CO₂ caused by changes of urban-rural and land-use boundaries.

Moreover, the well-identified poikilohydric natures of these organisms make them sensitive tracers of water vapor fluxes. Indeed, application of the stable oxygen isotope ratio (δ¹⁸O) to gain insights into the yet unknown fractionation processes of terrestrial poikilohydric organisms showed that thallus water isotopic composition is additionally influenced by the prevailing environmental conditions to which they are exposed to. Thus, we may use these results to assess the effect of a substantial lichen ground cover on water exchange processes between the soil and atmosphere. Numerous nutrient poor habitats in extreme climates are dominated by poikilohydric organisms. Hence, globally, the effects of these organisms on overall water fluxes may even be more remarkable.

Lichen communities can thus be used to study important interactions between atmosphere and biosphere. Accordingly, the concomitant application of geostatistical models and *lichen functional diversity* (*green algal and cyanobacterial LDV indexes*) are a suitable way to use lichen communities as good indicators of complex interactions between atmospheric nutrients deposition (i.e. atmospheric NH₃) and the biosphere, as well as microclimatic changes due to forestry and land-use practices.

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